Shape Shifting

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# Stereoselective Fluorination Alters the Geometry of a Cyclic Peptide: Exploration of Backbone-Fluorinated Analogues of Unguisin A**

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***Abstract:*** *New methods for enhancing the efficiency of peptide cyclization, and for fine-tuning the conformations of cyclic peptides, are valuable from a drug development perspective. Herein stereoselective fluorination is investigated as a new strategy for achieving these goals. Four vicinal difluorinated analogues of the natural cyclic heptapeptide unguisin A have been efficiently synthesized. The analogues are found to adopt dramatically different secondary structures, controlled by the fluorine stereochemistry.*

One of the outcomes of incorporating fluorine into organic molecules[1] is that the molecular conformation can be affected.[2] The highly polarized C–F bond engages in a variety of stereoelectronic interactions with neighboring functional groups, and such interactions can favor certain molecular conformations over others. For example, C–F bonds tend to align *gauche* to vicinal electronegative[3] or positively charged[4] substituents, due to a combination of hyperconjugative and electrostatic effects. Also, C–F bonds prefer to align antiparallel to adjacent carbonyl groups, which can be rationalized in terms of dipolar repulsion.[5] With a knowledge of such effects, it has become possible to rationally "program" molecules to adopt desired conformations, by decorating them with judiciously designed patterns of fluorine substituents.[2,6] This concept has been exploited to enhance the properties of a variety of functional molecules including organocatalysts,[7] liquid crystals,[8] enzyme inhibitors,[9] biological receptor ligands,[10] fatty acids,[11] and peptides.[12–16]

The latter context is of particular interest, because peptides are archetypal systems in which conformation determines function. Research into stereoselectively fluorinated peptides is in its infancy, but several groups have made important findings. Raines and co-workers[12] discovered that 4-fluoroproline increases the stability of the collagen triple helix when incorporated in place of natural hydroxyproline residues. Seebach and co-workers,[13] and Abell and co-workers,[14] have demonstrated that a single C–F bond can profoundly influence the secondary structure of β-peptides. O'Hagan and co-workers[15] have developed difluorosuccinate derivatives as shape-controlled components of symmetrical pseudopeptides. We have also made preliminary contributions in this area, by investigating the difluorinated γ-amino acids **1** and **2** (Figure 1);[16] these isomeric molecules have

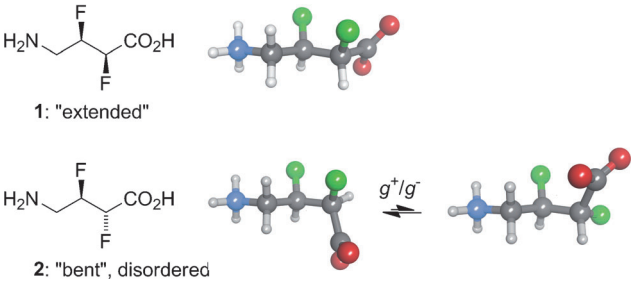


***Figure 1.*** Formulae and low-energy conformations of fluorinated amino acids **1** and **2**.

quite different conformations, consistent with the stereoelectronic effects described above. We have shown that the different conformations of **1** and **2** translate into different selectivity patterns in GABA receptor binding assays.[17,18] We have also shown that the different molecular shapes of **1** and **2** are preserved when these building blocks are incorporated into short peptides.[19,20]

To date, virtually all of the research on stereoselectively fluorinated peptides has focused on linear peptide systems. However, methods for fine-tuning the conformations of cyclic peptides will arguably offer richer possibilities in terms of biological and medicinal applications, because cyclic peptides usually have superior pharmacokinetic and pharmacodynamic properties relative to their linear counterparts, and are therefore regarded as better drug leads.[21] Controlling or altering the geometry of a cyclic peptide is a challenging problem, because this may entail competing against mechanical constraints inherent to the macrocycle itself. Accordingly, in this project we aimed to elaborate the fluorinated amino acids **1** and **2** into cyclic architectures in order to determine, for the first time,[22] whether selective fluorination can influence the conformation of a cyclic peptide.

We chose the natural product unguisin A (**3**, Scheme 1) as the scaffold for these investigations. Unguisin A is a marine-derived cyclic heptapeptide containing the rare structural feature of a GABA residue imbedded within the macrocycle.[23] We recently completed the first total synthesis of

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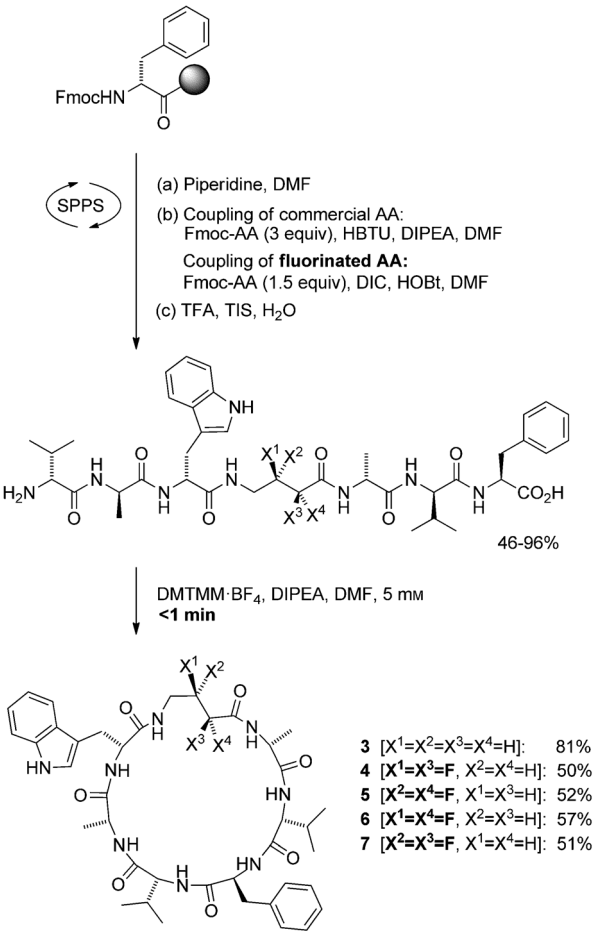


***Scheme 1.*** Synthesis of unguisin A (**3**)[24] and fluorinated analogues (**4**–**7**). AA: amino acid; Fmoc: fluorenylmethoxycarbonyl; HBTU: 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; DIPEA: *N*,*N*-diisopropylethylamine; DMF: dimethylformamide; DIC: *N*,*N*′-diisopropylcarbodiimide; HOBt: 1-hydroxybenzotriazole; TFA: trifluoroacetic acid; TIS: triisopropylsilane.

unguisin A with a macrocyclization approach (Scheme 1),[24] and so the new goal was to obtain analogues containing **1** or **2** (or the enantiomers of these fluorinated building blocks) in place of the GABA component. This ambition also raised an additional question: the building blocks **1** and **2** would give rise to differently shaped linear precursor peptides, and so it was of interest to determine whether these different shapes would manifest in different cyclization efficiencies.[25]

The requisite linear precursor peptides (Scheme 1) were readily assembled in good overall yields by solid-phase peptide synthesis,[26] employing the base-free conditions (DIC, HOBt) that were previously identified as optimal for peptide coupling steps involving fluorinated amino acids.[20] With the fluorinated linear precursor peptides in hand, attention was next turned to comparing their macrocyclization efficiencies. In the original synthesis of natural unguisin A,[24] macrocyclization was performed at room temperature using the coupling reagent DMTMM·$BF_4$[27] at a peptide concentration of 5 mM. These conditions delivered the target cyclic peptide **3** as the only observed product in excellent yield after only a short reaction time.[24] Accordingly, identical reaction conditions were employed for the macrocyclization reactions of the fluorinated analogues (Scheme 1). The reactions were performed on preparative scale and monitored at regular time intervals by LC–MS. To our surprise, all of the reactions had proceeded to completion by the time the first LC–MS traces were recorded, cleanly delivering the desired product in each case. Preparative HPLC subsequently afforded **4**–**7** all in very similar yields (Scheme 1), making it difficult to assess whether one cyclization was more efficient than another. In a further attempt to rank the efficiencies of cyclization, the reactions were repeated on analytical scale at 0 °C with continuous monitoring by $^1$H NMR spectroscopy.[28] However, once again the reactions all proceeded so rapidly and cleanly that only analytically pure products (**4**–**7**) were observed after one minute, the shortest time in which the reagents could be combined and a spectrum recorded. Thus, we were able to answer one of the two questions posed earlier, by concluding that the fluorine stereochemistry does not impact on the peptide cyclization efficiency in this system.[29]

Attention was next turned to the second question: can fluorination alter the secondary structure of a cyclic peptide? To answer this question, we performed a suite of NMR and molecular-modeling experiments to determine the preferred geometries of our chosen reference compound (**3**) and the fluorinated analogues (**4**–**7**). The $^1$H NMR spectra[30] of **3**–**7** were recorded at a range of temperatures, and the variations in the chemical shifts of individual signals[28,31] revealed that certain NH protons were involved in hydrogen bonding (Figure 2, left column, red coloring).

We next performed *J*-based analyses to further probe the molecular conformations. Compound **3** had previously been shown to exhibit conformational disorder in the GABA region, judging by the intermediate magnitude of the corresponding $^3J_{H,H}$ values.[24] The story does not change dramatically with the first *syn*-difluoro analogue, **4**: in this case, the $^3J_{H,H}$ and $^3J_{H,F}$ values about the difluoro axis are all intermediate in magnitude,[28,32] suggesting that the fluorine substituents are *gauche* but that there is $g^+/g^-$ disorder. A very different picture emerges for the other *syn*-difluoro analogue, **5**: in this case, the $^3J$ values about the difluoro axis are more extreme in magnitude,[28,32] indicating that an extended zigzag GABA structure with *gauche* fluorine substituents is strongly preferred. Notably, while the *syn*-difluoro isomers (**4**, **5**) are found to be quite different from one another, it emerges that the *anti*-difluoro isomers (**6**, **7**) seem very similar. For both of compounds **6** and **7**, the $^3J_{H,H}$ and $^3J_{H,F}$ values about the difluoro axis are small and intermediate in magnitude, respectively,[28,32] indicating that the fluorine substituents are *gauche* in each case but that there is $g^+/g^-$ disorder.

A final piece of information was obtained from the *J*-based analyses: $^4J_{H,F}$ couplings were not observed between the α-fluorine atom and the adjacent NH proton for any of the fluorinated analogues, suggesting that the expected[5] antiparallel C–F/C=O alignments were not realized.[33] This may be indicative of some strain imposed by the macrocyclic architecture.[34]

To assimilate all of the NMR-derived information described above, we used the Discovery Studio software package to perform conformer searches followed by energy

  

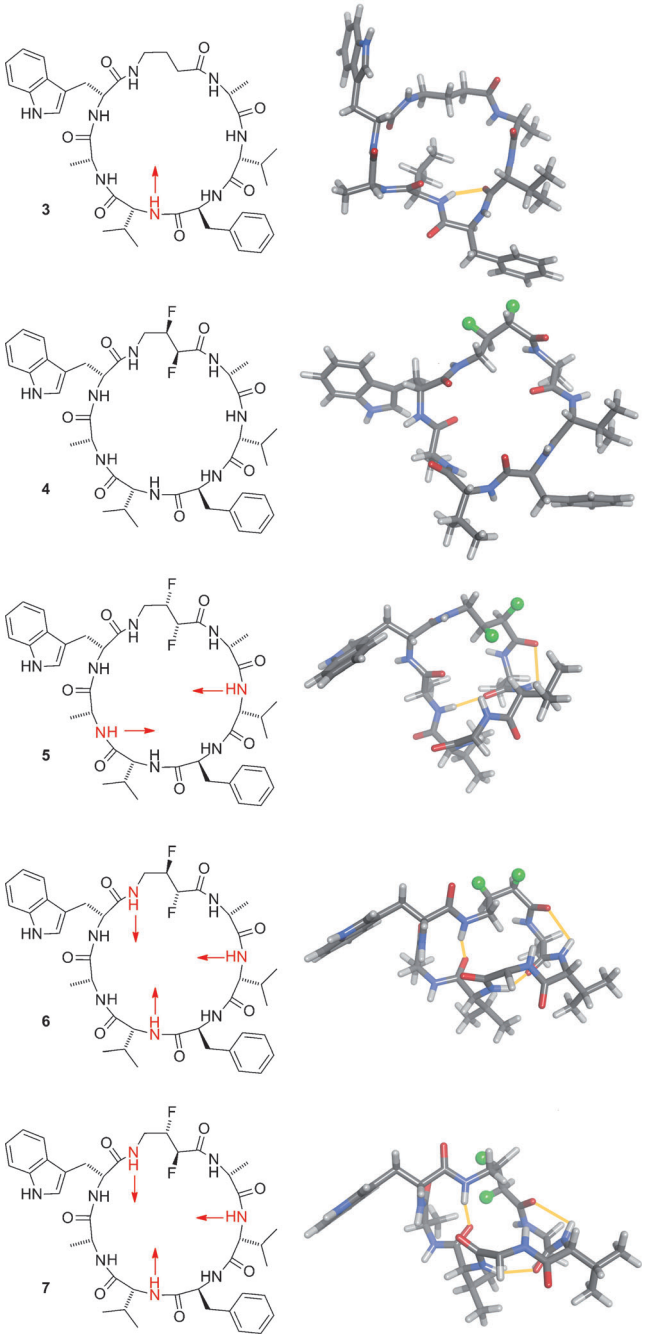


***Figure 2.*** Low-energy geometries of **3**–**7** were identified through a combination of NMR and molecular-modeling experiments. The fluorine atoms, which are depicted in the presentation on the right as green spheres, are *gauche* in every structure. The phenylalanine side chains of **5**–**7** are omitted for clarity. H-bonds are colored orange.

minimizations.[35] These experiments yielded multiple low-energy candidate geometries for **3**–**7**, from which final geometries were selected (Figure 2, right) based on their agreement with the NMR data described above.[36] This process revealed that stereoselective fluorination can indeed alter the geometry of a cyclic peptide. The nonfluorinated reference compound (**3**) adopts a predominantly flat conformation, with just one pucker in the southwestern portion of the molecule constituting an equatorial γ-turn (that is, a seven-membered H-bonded ring).[37] All four of the fluorinated analogues (**4**–**7**) have geometries that are quite different from that of **3**. The first *syn*-difluoro analogue, **4**, has a fully planar geometry with no hydrogen-bonded turn structures, whereas the other *syn*-difluoro analogue, **5**, has a geometry that is more puckered than that of the reference compound, **3**. Analogue **5** contains two overlapping hydrogen-bonded loops: an equatorial γ-turn and a distorted α-turn (that is, a 13-membered H-bonded ring).[37] Finally, the two *anti*-difluoro analogues (**6**, **7**) have geometries that are very similar to one another, but quite different from what has come before. Both analogues have highly compressed and puckered structures, featuring two β-turns (that is, ten-membered H-bonded rings),[37] one of which overlaps with an additional γ-turn.

Overall, it is striking that the *syn*-difluoro analogues (**4**, **5**) have very different conformations, while the *anti*-difluoro analogues (**6**, **7**) are very similar to one another. This seems to parallel the results of a previous GABA receptor binding study[18] involving the free amino acids **1** and **2** (Figure 1): the *syn* isomers (**1** and *ent*-**1**) displayed opposite pharmacology at the $GABA_C$ receptor, whereas the *anti* isomers (**2** and *ent*-**2**) displayed identical activity at the $GABA_A$ receptor. An explanation can now be offered to unify the observations of that study[18] and the present one. By coincidence, the lowest-energy geometry of **2** (Figure 1) closely resembles the next-higher-energy geometry of *ent*-**2**, and vice versa, provided fluorine is assumed to be a reasonable mimic of hydrogen. Since **2** and *ent*-**2** exhibit $g^+/g^-$ disorder, they both populate the same two backbone geometries. This "pseudo-homomeric" relationship also manifests in the structures of cyclic peptides **6** and **7** (Figure 3): two low-energy geometries of **6** closely resemble two low-energy geometries of **7**, with the only difference being the orientations of the fluorine substituents.[38] Critically, the lowest- and next-higher-energy conformers of the *syn*-difluoro compounds do not have this pseudo-homomeric relationship,[18,19] and so it follows that the *syn*-difluoro cyclic peptides **4** and **5** (and the *syn*-difluoro GABA receptor ligands **1** and *ent*-**1**) behave as genuinely different compounds.

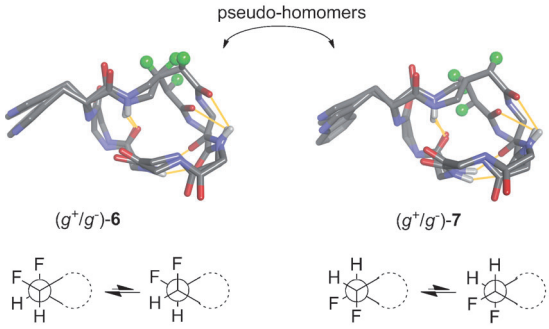


***Figure 3.*** Overlaying the $g^+/g^-$ conformers of **6** and **7** illustrates the pseudo-homomeric relationship between these two compounds. All side chains except Trp are omitted for clarity.

In conclusion, we have demonstrated for the first time that stereoselective fluorination can alter the geometry of a cyclic

  

peptide. This represents a new strategy for fine-tuning the conformations of this biologically important class of molecules, which may inform the ongoing development of other cyclic peptide drug leads towards optimal potency and selectivity.

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